

IN THE CLAIMS

Amend the claims as follows.

1. (Previously Amended) A method for purifying plasmid DNA from a mixture of same containing at least one host cell impurity comprising the following steps:
 - (a) forming a solution with said mixture wherein said solution has a salt concentration in the range of about 2M to 4M to allow selective binding of said at least one host cell impurity to a hydrophobic interaction media;
 - (b) contacting said solution containing plasmid DNA with said hydrophobic interaction media under conditions that said at least one impurity binds to the hydrophobic interaction media to form a complex; and
 - (c) collecting unbound plasmid DNA from said complex;wherein said method is conducted in the absence of organic solvents, detergents, glycols, hexamine cobalt, spermidine, and polyvinylpyrrolidone.
2. (Original) The method of claim 1 wherein the at least one impurity is selected from the group consisting of RNA, endotoxin, chromosomal DNA and protein.
3. (Original) The method for claim 1 wherein the at least one impurity is an endotoxin.

4. (Original) The method of claim 1 wherein the salt comprises an anion or cation selected from the group consisting of acetate, phosphate, carbonate, SO_4^{2-} , Cl^- , Br^- , NO_3^- , Mg^{2+} , Li^+ , Na^+ , K^+ and NH_4^+ .
5. (Previously Amended) The method of claim 4 wherein the salt is ammonium sulfate.
6. (Original) The method of claim 5 wherein ammonium sulfate is present at a concentration of about 2M.
7. (Previously Amended) The method of claim 1 wherein the solution comprises sodium salts in a concentration range of about 2M to 4M.
8. (Original) The method of claim 7 wherein the sodium salt is sodium chloride.
9. (Original) The method of claim 8 wherein the sodium salt is sodium chloride in a concentration of about 2M.
10. (Original) The method of claim 1 wherein the pH of the solution has a range of about 6.8 to about 7.4.
11. (Original) The method of claim 1 wherein the pH of the solution is about 7.4.

12. (Original) The method of claim 1 wherein the hydrophobic interaction media comprises a chromatography support with pendent hydrophobic groups.

13. (Original) The method of claim 12 wherein said pendent groups are selected from the group consisting of C₃ to C₁₀ alkyl groups and mixtures thereof.

14. (Previously Amended) The method of claim 12 wherein the hydrophobic interaction media are selected from the group consisting of a methacrylate polymer or copolymer backbone bound to a least one of a propyl, butyl, hexyl, octyl, nonyl or decyl ligand.

15. (Previously Amended) The method of claim 12 wherein the media is at least one of a methacrylate ethylene glycol copolymer backbone or a cross-linked agarose backbone.

16. (Previously Amended) The method of claim 12 wherein the support is in the form of bead in the size range of 15 to 100 μm .

17. (Previously Amended) A method of separating supercoiled plasmid DNA from a mixture of supercoiled plasmid DNA and relaxed plasmid DNA and, optionally, at least one host cell impurity comprising the following steps:

(a) forming a solution by adding a salt to the mixture of supercoiled plasmid DNA and relaxed plasmid DNA and, when present, said at least one host cell impurity;

(b) contacting the solution with a hydrophobic interaction media under a first condition where both the supercoiled plasmid DNA and relaxed plasmid DNA bind to the hydrophobic interaction media to form a bound first mixture;

(c) altering the first condition surrounding the bound first mixture to a second condition to remove relaxed plasmid DNA from the bound first mixture to form separate components containing a second bound mixture and relaxed plasmid DNA; and

(d) modifying the second condition surrounding the said second bound mixture to a third condition to remove supercoiled plasmid DNA from said second bound mixture to form separate components containing hydrophobic interaction media and supercoiled plasmid DNA.

18. (Original) The method of claim 17 wherein the at least one host cell impurity is selected from the group consisting of RNA, endotoxin, chromosomal DNA and protein.

19. (Original) The method for claim 17 wherein the at least one host cell impurity is an endotoxin.

20. (Original) The method of claim 17 wherein the hydrophobic interaction media comprises a chromatography support with pendent hydrophobic groups.

21. (Original) The method of claim 20 wherein said pendent groups are selected from the group consisting of C₃ to C₁₀ alkyl groups.

22. (Original) The method of claim 20 wherein the hydrophobic interaction media is selected from the group consisting of a methacrylate polymer or copolymer backbone bound to a least one of a propyl, butyl, hexyl, octyl, nonyl, or a mixture of these as ligands.

23. (Original) The method of claim 20 wherein the media is at least one of a methacrylate ethylene glycol copolymer backbone or a cross-linked agarose.

24. (Original) A method of claim 20 wherein the media is a resin in the form of beads in the size range of 15 to 100 μm .

25. (Original) The method of claim 17 wherein the salt comprises an anion or cation selected from the group consisting of acetate, phosphate, carbonate, SO_4^{2-} , Cl^- , Br^- , NO_3^- , Mg^{2+} , Li^+ , Na^+ , K^+ and NH_4^+ .

26. (Original) The method of claim 25 wherein the salt is ammonium sulfate in a concentration range of 2.5M to 4M.

27. (Previously Amended) The method of claim 17 wherein the first condition comprises equilibrating said media with a salt solution containing ammonium sulfate which is present in a concentration range of about 2.5M to 4M.

28. (Previously Amended) The method of claim 17 wherein the second condition comprises washing the media with a salt solution containing ammonium sulfate in a concentration of about 2.35M to about 2.45M.

29. (Previously Amended) The method of claim 17 wherein the said third condition comprises washing said second bound mixture with a salt solution containing ammonium sulfate in a concentration of about 1M to 2.3M.

Claims 30-40 (Canceled).

41. (Previously Amended) A method of separating supercoiled plasmid DNA from relaxed plasmid DNA comprising contacting a mixture of supercoiled plasmid DNA and relaxed plasmid DNA with a hydrophobic interaction media under a first condition where both the supercoiled plasmid DNA and the relaxed plasmid DNA bind to said hydrophobic interaction media to form a bound first mixture, altering said first condition surrounding the bound first mixture to a second condition to remove said relaxed plasmid DNA from said bound first mixture to form separate components containing a second bound mixture and said relaxed plasmid DNA, and modifying the second

condition surrounding said second bound mixture to a third condition to remove said supercoiled plasmid DNA from said second bound mixture to form separate components containing said hydrophobic interaction media and said supercoiled plasmid DNA.

42. (Original) The method of claim 41 wherein said hydrophobic interaction media comprises a chromatographic support with pendent hydrophobic groups.

43. (Original) The method of claim 42 wherein said pendent hydrophobic groups are selected from the group consisting of C₃ to C₁₀ alkyl groups and mixtures thereof.

44. (Currently Amended) The method of claim 41 wherein said hydrophobic ~~resin-~~ interaction media is a methacrylate polymer or copolymer backbone bound to at least one of a propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, or decyl ligand.

45. (Currently Amended) The method of claim 41 wherein said ~~resin~~ hydrophobic interaction media is at least one of a methacrylate and ethylene glycol copolymer backbone or a cross-linked agarose.

46. (Currently Amended) The method of claim 41 wherein said ~~support~~ hydrophobic interaction media is in the form of beads ranging in size from 15 to 100 μm .

47. (Previously Amended) The method of claim 41 wherein said first condition comprises equilibrating said media with a salt solution containing ammonium sulfate in a concentration range of about 2.5 M to about 4 M.

48. (Previously Amended) The method of claim 47 wherein said second condition comprises washing said bound first mixture with a salt solution containing ammonium sulfate in a concentration of about 2.35 M to about 2.45 M.

49. (Previously Amended) The method of claim 48 wherein said third condition comprises washing said second bound mixture with a salt solution containing ammonium sulfate in a concentration of about 1 M to about 2.3M.

50. (Original) The method of any one of claims 17 and 41 wherein said altering and said modifying are combined in a continuous process comprising gradient elution of said relaxed plasmid DNA and supercoiled plasmid DNA by mixing said bound first mixture with an ammonium sulfate containing salt solution with a continuously varying concentration of ammonium sulfate, said concentration varying from about 3M to about 1 M ammonium sulfate, and said relaxed plasmid DNA is collected in a first eluted volume and said supercoiled plasmid DNA is collected in a second eluted volume.

51. (Original) The method of claim 41 wherein said separate relaxed plasmid DNA component and said separate supercoiled plasmid DNA are collected and isolated.

Claims 52-53 (Canceled).

54. (Previously Added) A method of enriching supercoiled DNA relative to relaxed DNA in a mixture thereof, the method comprising :

(a) forming a solution by adding a salt to the mixture of supercoiled DNA and relaxed DNA;

(b) contacting the solution with a hydrophobic interaction media under a first condition where both the supercoiled DNA and relaxed DNA bind to the hydrophobic interaction media to form a bound first mixture;

(c) altering the first condition surrounding the bound first mixture to a second condition to remove relaxed DNA from the bound first mixture to form separate components containing a second bound mixture and relaxed DNA; and

(d) modifying the second condition surrounding the said second bound mixture to a third condition to remove supercoiled DNA from said second bound mixture to form separate components containing hydrophobic interaction media and supercoiled DNA.

55. (Previously Added) The method of claim 54 wherein the hydrophobic interaction media comprises a chromatography support with pendent hydrophobic groups.

56. (Previously Added) The method of claim 55 wherein said pendent groups are selected from the group consisting of C₃ to C₁₀ alkyl groups.

57. (Previously Added) The method of claim 55 wherein the hydrophobic interaction media is selected from the group consisting of a methacrylate polymer or copolymer backbone bound to a least one of a propyl, butyl, hexyl, octyl, nonyl, or a mixture of these as ligands.

58. (Previously Added) The method of claim 55 wherein the media is at least one of a methacrylate ethylene glycol copolymer backbone or a cross-linked agarose.

59. (Previously Added) A method of claim 55 wherein the media is a resin in the form of beads in the size range of 15 to 100 μm .

60. (Previously Added) The method of claim 54 wherein the salt comprises an anion or cation selected from the group consisting of acetate, phosphate, carbonate, SO₄²⁻, Cl⁻, Br⁻, NO₃⁻, Mg²⁺, Li⁺, Na⁺, K⁺ and NH₄⁺.

61. (Previously Added) The method of claim 60 wherein the salt is ammonium sulfate in a concentration range of 2.5M to 4M.

62. (Previously Added) The method of claim 54 wherein the first condition comprises equilibrating said media with a salt solution containing ammonium sulfate which is present in a concentration range of about 2.5M to 4M.

63. (Previously Added) The method of claim 54 wherein the second condition comprises washing the media with a salt solution containing ammonium sulfate in a concentration of about 2.35M to about 2.45M.

64. (Previously Added) The method of claim 54 wherein the said third condition comprises washing said second bound mixture with a salt solution containing ammonium sulfate in a concentration of about 1M to 2.3M.